Appendix B

Blind Audit Report

Chesapeake Bay Program

Blind Audit Nutrient Results

January and June 1998

November 1998

Carl Zimmermann
Carolyn Keefe
Nutrient Analytical Services Laboratory
Chesapeake Biological Laboratory
Solomons, MD 20688

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INTRODUCTION:

The purpose of this Blind Audit Program is to provide samples of specific nutrient analytes at concentrations commonly found in estuarine systems for analysis by laboratories who analyze water samples collected from the Chesapeake Bay and its tributaries. The concentrations of these samples, which are unknown to the recipient analysts, are compared to their true concentrations.

In the early years of the Chesapeake Bay Program, the U.S. EPA provided blind audit samples on an irregular basis to laboratories analyzing Chesapeake Bay water samples. However, these audit samples were designed for waste water/drinking water applications rather than estuarine water applications. Consequently, the concentrations were much higher than normally occur in the Bay and did not provide a reasonable estimate of accuracy for low level nutrient analyses. For example, a blind audit concentration of 1.0 mg NH4-N/L would be comparable for NPDES water samples but would be an order of magnitude greater than concentrations normally occurring in most parts of Chesapeake Bay.

The only continuous program providing an estimate of laboratory performance has been the Chesapeake Bay Coordinated Split Sample Program (CSSP). Data generated from this program provide the only long term QA/QC data base that compare nutrient measurements provided by laboratories analyzing water samples collected from Chesapeake Bay and its tributaries. Samples for the CSSP are natural water samples collected from Chesapeake Bay or a tributary. Briefly, a common unfiltered water sample is distributed to the various field/laboratory personnel who in turn subsample into dissolved and particulate fractions. These are analyzed and the results compared to those of other participating laboratories. Resulting data analysis can show how field filtration techniques and/or laboratory practices affect data variability. The CSSP samples are each subject to cumulative errors of analytical determinations from variation in both field and laboratory procedures. Also, these data sets cannot definitively determine the accuracy of laboratory analyses.

The current Blind Audit Program was designed to complement the CSSP. Blind Audit particulate samples distributed to participants have few cumulative errors associated with field filtering and subsampling procedures. Prepared concentrates of dissolved substances, whose concentrations are unknown to the analysts, are provided so that laboratory accuracy can be assessed.

There have been no blind audit assessments within the Chesapeake Bay Program for the past nine years. It is the intent of this Blind Audit Program to continually provide unknown, low level dissolved and particulate nutrient samples to laboratories analyzing Chesapeake Bay Program nutrients, as well as to other laboratories interested in participating in the Blind Audit Program.

MATERIALS AND METHODS

Blind Audit samples were sent to participating laboratories in January (27 January 1998) and June (15 June 1998) 1998. Those participating laboratories and contact personnel are found in Table 1.

Parameters measured during the January audit were: total dissolved nitrogen, total dissolved phosphorus, nitrate+nitrite, ammonium and phosphate. A high and a low concentration sample were provided for each of these analytes. Particulate carbon, nitrogen and phosphorus samples were also provided for those laboratories that routinely analyze these parameters.

Dissolved Blind Audit concentrates were prepared by careful dilution of high quality standards using 18.3 megohm deionized water. The concentrates were sealed in 10 mL ampules for shipment to the participants. One ampule contained a concentrate of an organic nitrogen compound and an organic phosphorus compound to be diluted for the analysis of low level total dissolved nitrogen and total dissolved phosphorus. A second ampule contained a concentrate of organic nitrogen and organic phosphorus to be diluted for the analysis of higher level total dissolved nitrogen and total dissolved phosphorus. A third ampule contained a concentrate to be diluted for the analysis of low level inorganic nutrients (ammonium, nitrate and phosphate). A fourth ampule contained a concentrate to be diluted for

the analysis of higher level inorganic nutrients. At each participating laboratory, an aliquot from each ampule was diluted and analyzed according to accompanying instructions for preparation and dilution. Blind Audit samples were then inserted randomly in a typical estuarine sample set. Final concentrations were reported for each diluted concentrate according to the dilution instructions provided.

Particulate analytes are measured by analyzing suspended material concentrated on filter pads. There are no commercially available suspensions of pure carbon, nitrogen or phosphorus compounds, so a natural sample was subsampled onto filter pads for analysis by participating laboratories. A batch water sample was collected off the CBL pier in January and June, and subsampled for particulate samples of carbon, nitrogen and phosphorus. Particulate C/N samples were filtered from the batch sample with care being taken to shake the sample before each filtration to ensure homogeneity. Four 25 mm GF/F pads were sent to each laboratory for analysis. One laboratory s instrument requires that only 13 mm filters be utilized. For that laboratory, four 13 mm GF/F pads were provided. Samples were dried completely (overnight at 47°C) before shipment. Vacuum filtration was used to process the 25 mm filters, but positive pressure was used to filter the 13 mm filters. Our laboratory did not have the facilities necessary to vacuum filter these small filters.

The same general procedure was followed for particulate phosphorus samples which were concentrated by vacuum filtration on 47 mm GF/F pads.

Particulate concentrations for the January Blind Audit were estimated as closely as possible by analyzing at least eight replicates of each analyte by Chesapeake Biological Laboratory. These calibration replicates also provided an estimate of variability due to the cumulative effect of filtering and other processing errors. Filter pads were sent to each laboratory for the analysis of particulate C, N and P. The volume of sample filtered was noted in the instructions so that each laboratory could report values in mg/L.

For the June Blind Audit, two samples concentrated on filters were supplied to each laboratory for each particulate analysis. One laboratory analyzed a second pair of filters because the first pair was rejected when the analyst noticed a marked visible difference between the replicates. The standard deviations determined for the January particulate fractions also were used to assess the variability of the June data.

Analysis of chlorophyll *a* samples was added to the suite of nutrients in June 1998. Samples were filtered onto 47 mm GF/F glass fiber filters and two were then sent to each laboratory.

For both audits, samples were sent in coolers via next day carrier to the participating laboratories. In June, when chlorophyll samples were sent, a cold temperature was required, so frozen cold packs were packed in those coolers.

RESULTS

JANUARY 1998 DISSOLVED FRACTION

Figures summarizing all results are found at the end of the report.

Total Dissolved Nitrogen: The true low level concentration was 0.35 mg N/L and reported concentrations ranged from 0.27-0.40 mg N/L. The true high level concentration was 1.05 mg N/L and reported concentrations ranged from 0.97-1.15 mg N/L. All laboratories reported concentrations that were within 0.10 mg N/L of the respective total dissolved nitrogen concentrations.
Total Dissolved Phosphorus: The true low level concentration was 0.024 mg P/L and reported concentrations ranged from 0.020-0.040 mg P/L. The true high level concentration was 0.096 mg P/L and reported concentrations ranged from 0.050-0.110 mg P/L.. All laboratories except one reported concentrations within 0.005 mg P/L of the true concentration for the low level total dissolved phosphorus sample. All laboratories except one reported concentrations within 0.015 mg P/L of the true concentration

for the higher level total dissolved phosphorus concentration.

Ammonium: The true low level concentration was 0.063 mg N/L and reported concentrations ranged from 0.060-0.081 mg N/L. The true high level concentration was 0.330 mg N/L and reported concentrations ranged from 0.320-0.364 mg N/L. All laboratories except one reported concentrations within 0.006 mg N/L of the true low level ammonium concentration. All laboratories reported concentrations within 0.034 mg N/L of the true higher level ammonium concentration.

Nitrate+nitrite: The true low level concentration was 0.112 mg N/L and reported concentrations ranged from 0.110-0.126 mg N/L. The true high level concentration was 1.15 mg N/L and reported concentrations ranged from 1.12-1.23 mg N/L.. All laboratories reported concentrations within 0.014 mg N/L of the true low level nitrate concentration, and within 0.08 of the true higher level nitrate concentration.

Phosphate: The true low level concentration was 0.031 mg P/L and reported concentrations ranged from 0.020-0.040 mg P/L. The true high level concentration was 0.310 mg P/L and reported concentrations ranged from 0.298-0.335 mg P/L. All laboratories except two reported concentrations within 0.003 mg P/L of the true low level phosphate concentration. All laboratories reported concentrations within 0.025 mg P/L of the true higher level phosphate concentration.

JANUARY 1998 PARTICULATE FRACTION

Again, it should be noted that these samples were filtered from a common water sample and, consequently, are not true blind audit samples made from pure constituents; rather, a concentration range around a mean was established by the analysis of 12 replicate particulate C/N samples and 8 replicate particulate phosphorus samples. This still provides a verification of measurement processes in routine analytical conditions at participating laboratories, without the potential variability associated with differing field filtration techniques.

Particulate Nitrogen: The mean concentration of the 12 replicate samples was 0.078 mg N/L ± 0.004 (S.D.) and all but one of the responding laboratories reported the mean concentration of their four replicates within 0.078 mg N/L ± 0.012, i.e., 3 X S.D. .

Particulate Carbon: The mean concentration of the 12 replicate samples was 0.411 mg C/L ± 0.050 (S.D.) and all responding laboratories reported the mean concentration of their four replicates within 0.411 mg C/L ± 0.150, i.e., 3 X S.D..

Particulate Phosphorus: The mean concentration of the 8 replicate samples was 0.0318 mg P/L ± 0.0010 (S.D.) and all responding laboratories reported the mean concentration of their four replicates within 0.0318 mg P/L ± 0.0030, i.e., 3 X S.D..

JUNE 1998 DISSOLVED FRACTION

The concentrations of some Blind Audit samples were reduced for the June audit. Low level total dissolved N and P concentrations remained unchanged from the January concentrations, but the higher level concentrations were halved from those of January. Low level ammonium concentrations were also halved, as were the low level phosphate concentrations. The higher level concentration phosphate samples were reduced by a factor of five from the June samples. Basically, for the June Blind Audit, the true concentrations remained unchanged or were substantially reduced from January levels.

Total Dissolved Nitrogen: The true low level concentration was the same as in January, 0.35 mg N/L and reported concentrations ranged from 0.205-0.42 mg N/L. The true high level concentration was 0.53 mg N/L and reported concentrations ranged from 0.39-0.62 mg N/L. All laboratories reported concentrations

within 0.15 mg N/L of the true concentration of the respective total dissolved nitrogen concentrations.

Total Dissolved Phosphorus: The true low level concentration was 0.024 mg P/L (the same as January) and reported concentrations ranged from 0.020-0.030 mg P/L. The true high level concentration was 0.048 mg P/L and reported concentrations ranged from 0.030-0.0513 mg P/L. All laboratories reported concentrations within 0.006 mg P/L of the true low level total dissolved phosphorus concentration. All laboratories except one reported concentrations within 0.006 mg P/L of the true higher level total dissolved phosphorus concentration.

Ammonium: The true low level concentration was 0.035 mg N/L and reported concentrations ranged from 0.025-0.040 mg N/L. The true high level concentration was 0.280 mg N/L and reported concentrations ranged from 0.2645-0.281 mg N/L. All laboratories reported concentrations within 0.010 mg N/L of the true low level ammonium concentration, and within 0.020 of the true higher level ammonium concentration.

Nitrate+nitrite: The true low level concentration was 0.175 mg N/L and reported concentrations ranged from 0.160-0.210 mg N/L. The true high level concentration was 0.600 mg N/L and reported concentrations ranged from 0.550-0.594 mg N/L. All laboratories except one reported concentrations within 0.015 mg N/L of the true low level nitrate concentration. All laboratories reported concentrations within 0.050 mg N/L of the true higher level nitrate concentration.

Phosphate: The true low level concentration was 0.0186 mg P/L and reported concentrations ranged from 0.0190-0.0203 mg P/L. The true high level concentration was 0.0620 mg P/L and reported concentrations ranged from 0.0600-0.0672 mg P/L. All laboratories reported concentrations within 0.0020 mg P/L of the true low level phosphate concentration, and within 0.0060 of the true higher level phosphate concentration.

JUNE 1998 PARTICULATE FRACTION

Particulate Nitrogen: The mean concentration of the samples analyzed by the five participating laboratories was 0.307 mg N/L. Each reported mean from any participating laboratory was within 0.307 mg N/L $\pm$ 0.012, i.e., 3 X S.D. of the 12 January calibration replicates.

Particulate Carbon: The mean concentration of the samples analyzed by the five participating laboratories was 1.60 mg C/L. Each reported mean from any participating laboratory was within 1.60 mg C/L $\pm$ 0.15, i.e., 3 X S.D. of the 12 January calibration replicates.

Particulate Phosphorus: The mean concentration of the samples analyzed by the five participating laboratories was 0.0454 mg P/L.. Each reported mean from any participating laboratory was within 0.0454 mg P/L $\pm$ 0.0030, i.e., 3 X S.D. of the 8 January calibration replicates.

Chlorophyll: There was quite large variation between laboratories in the chlorophyll *a* concentrations reported. CBL and DCLS reported nearly identical concentrations, while the Academy of Natural Sciences was more than 7 μg/L greater, and VIMS and ODU reported concentrations substantially lower.

DISCUSSION

Three important issues should be considered when assessing whether individual Blind Audit results are within acceptable limits.

Variation Associated With An Analytical Method: A certain amount of analytical variability is associated with any quantitative determination. The method detection limit (three times the standard deviation of seven low level replicate natural samples) is often used to express that level of variation. Total dissolved

nitrogen data provide a good example. The detection limit at CBL has been determined to be 0.02 mg N/L. Any total dissolved nitrogen measurement has a potential 0.02 mg N/L variability associated with it. This variability, when expressed as a percent of the true concentration, can be extremely large for low level concentrations and fairly low for higher concentrations. For example, a 0.20 mg N/L concentration has an analytical variability of 10% associated with it; whereas, a 1.20 mg N/L concentration has an analytical variability of 2%.

Reporting Significant Figures: The number of significant figures used by a laboratory to report analytical results can significantly affect data interpretation in a blind audit study. If a laboratory reports only two significant figures (for whatever reasons) and an audit sample has a true concentration expressed in three significant figures, then substantial under or over estimates of the true concentration can be reported. For example, if a true value of 0.035 mg P/L has been prepared and a laboratory only reports two significant figures, i.e., 0.03 mg P/L, then the results expressed are 86% of the expected true value.

Preparation of True Standards: Companies that prepare large quantities of unknowns assign acceptable confidence limits around the true value. In one case (SPEX, CertiPrep), the mean recovery and standard deviation are later reported along with the true concentration and the 95% confidence interval (CI). The 95% CI represents the mean recovery $\pm$ 2 standard deviations and was developed from regression equations from Water Pollution Performance Evaluation Studies. A recently purchased set of these standards gave a true total P value of 3.00 mg P/L with a 95% CI of 2.47-3.42 mg P/L.. The lower end of the 95% CI recovery allows 82% recovery of the true concentration. This type of statistical analysis was not performed on the Blind Audit Program samples prepared for this study.

With the above issues in mind and even though only two rounds of the Blind Audit Program have been completed, some consistent patterns have been observed that warrant discussion or further investigation:

1. Reported concentrations of all analytes except total dissolved phosphorus and chlorophyll *a* are similar between laboratories participating in the Blind Audit Program. Except for total dissolved phosphorus, no laboratory reported concentrations for an individual analyte that were consistently different from the range of the other reported concentrations. This probably indicates that all participating laboratories execute these measurements with accuracy and precision.

2. If possible, all participants should report data from future Blind Audits to three significant figures to facilitate concentration comparisons.

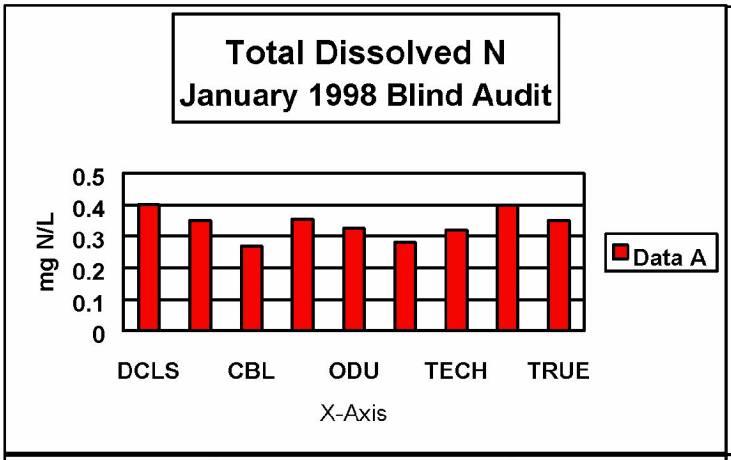
3. A 95% Confidence Interval for each concentration level of every analyte should be established, possibly with the assistance of EPA statisticians.

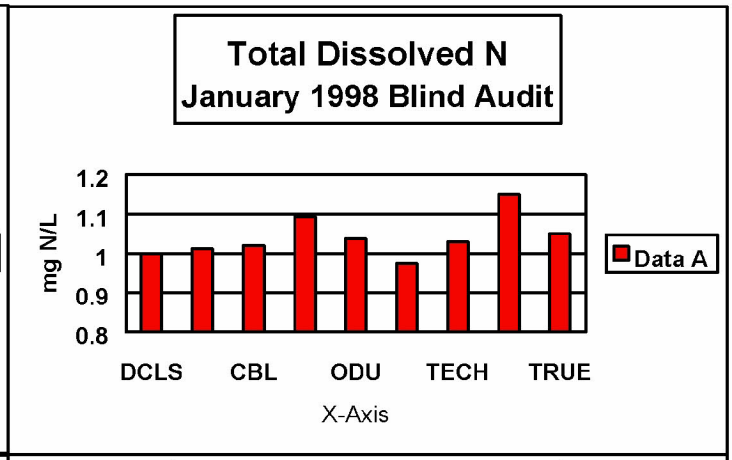
4. One laboratory reported consistently lower concentrations for total dissolved phosphorus in both the low and higher level samples. Although other laboratories reported concentrations for the low level sample that were similar, none reported similar concentrations for the higher level samples.

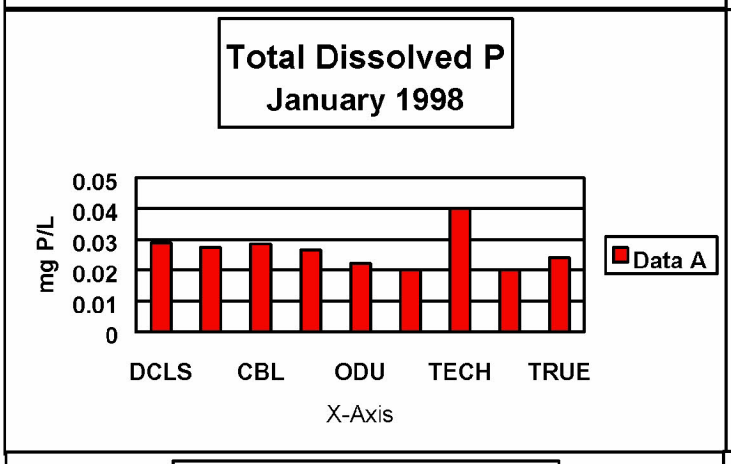
5. Reported chlorophyll *a* concentrations were quite variable. In connection with these data and other CBP chlorophyll *a* data anomalies, the CBP Quality Assurance Officer is contacting all participants with respect to methodology--spectrophotometric-one wave length/trichromatic/fluorometric; type of grinding; use of buffers; etc.

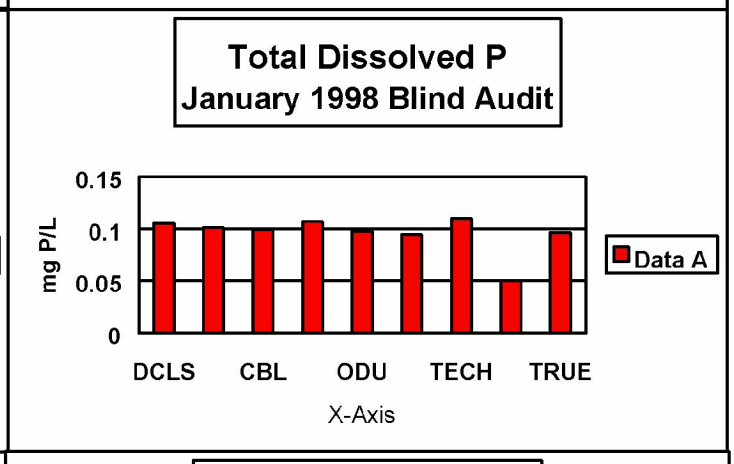
Table 2 lists concentrations of analytes where the difference between the reported concentration and the true concentration was more than two times a typical MDL in both the January and June Blind Audits. These differences may not be cause for concern since 95% confidence intervals have not been assigned.

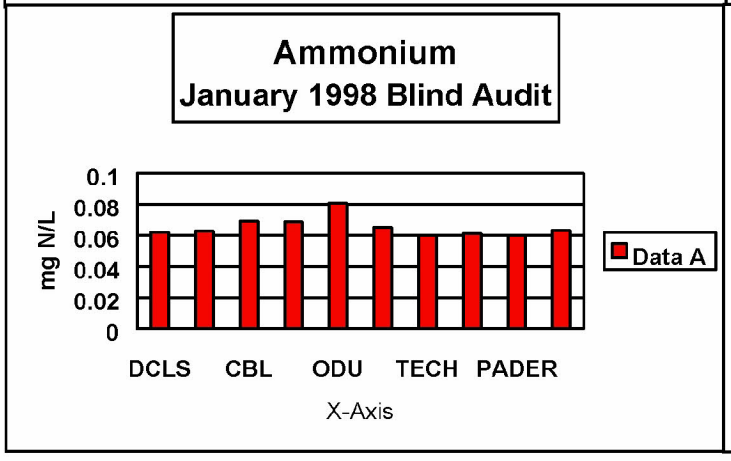
Table 2. Consistent differences noted in 1998 Blind Audit results							
Total Dissolved Nitrogen; Low Concentration (mg N/L)							
January					June		
Lab.	True	Reported	% of True		True	Reported	% of True
CBL	0.35	0.27	77%		0.35	0.30	86%
HPL	0.35	0.281	80%		0.35	0.205	59%
PADER	0.35	0.40	114%		0.35	0.42	120%
Total Dissolved Nitrogen; High Concentration (mg N/L)							
January					June		
Lab.	True	Reported	% of True		True	Reported	% of True
PADER	1.05	1.15	109%		.53	.62	117%
Total Dissolved Phosphorus; Low Concentration (mg P/L)							
January					June		
Lab.	True	Reported	% of True		True	Reported	% of True
CBL	.024	.0285	119%		.024	.0205	85%
HPL	.024	.020	83%		.024	.021	87%
PADER	.024	.02	83%		.024	.02	83%
Total Dissolved Phosphorus, High Concentration (mg P/L)							
January					June		
Lab.	True	Reported	% of True		True	Reported	% of True
PADER	.096	.05	52%		.048	.03	62%

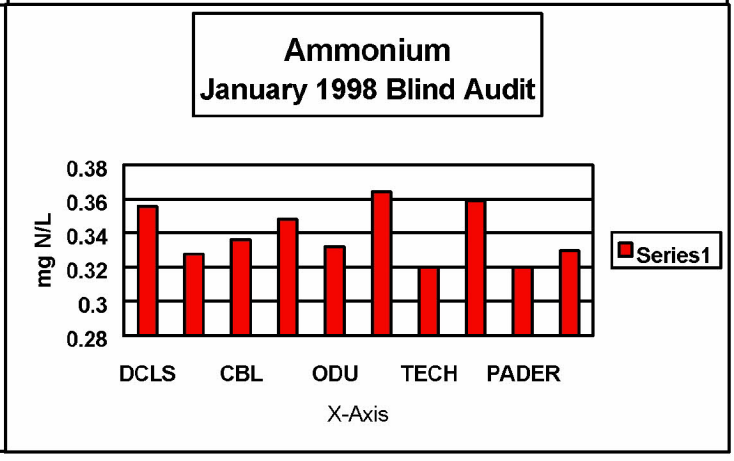

Total Dissolved N
January 1998 Blind Audit
mg N/L
0.5
0.4
0.3
0.2
0.1
0
DCLS
CBL
ODU
TECH
TRUE
X-Axis
Data A

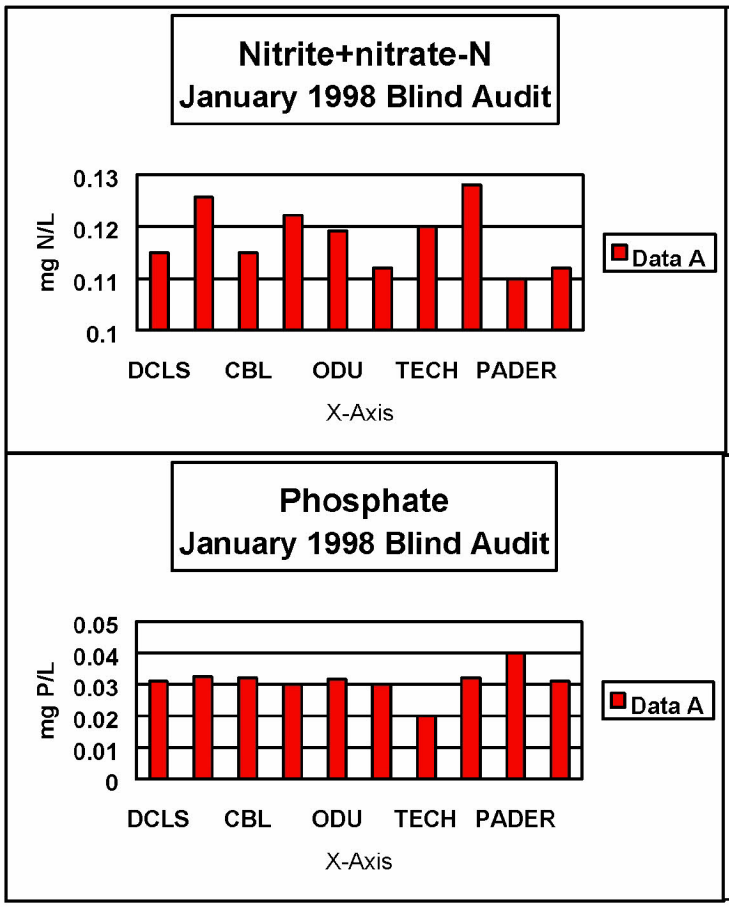

Total Dissolved N
January 1998 Blind Audit
mg N/L
1.2
1.1
1
0.9
0.8
DCLS
CBL
ODU
TECH
TRUE
X-Axis
Data A

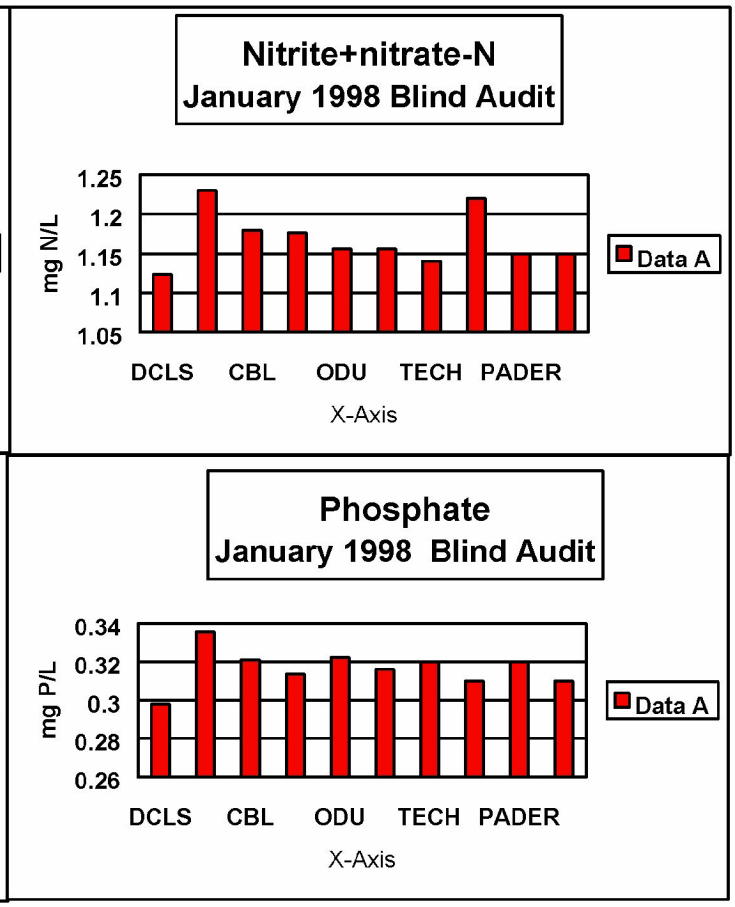

Total Dissolved P
January 1998
mg P/L
0.05
0.04
0.03
0.02
0.01
0
DCLS
CBL
ODU
TECH
TRUE
X-Axis
Data A


Total Dissolved P
January 1998 Blind Audit
mg P/L
0.15
0.1
0.05
0
DCLS
CBL
ODU
TECH
TRUE
X-Axis
Data A


Ammonium
January 1998 Blind Audit
mg N/L
0.1
0.08
0.06
0.04
0.02
0
DCLS
CBL
ODU
TECH
PADER
X-Axis
Data A

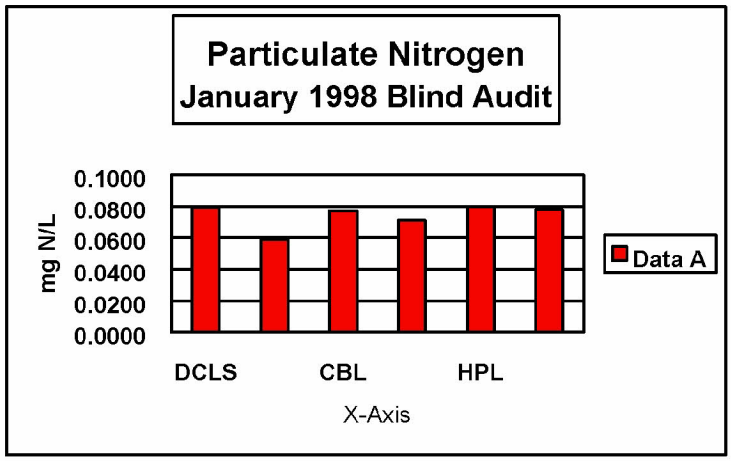

Ammonium
January 1998 Blind Audit
mg N/L
0.38
0.36
0.34
0.32
0.3
0.28
DCLS
CBL
ODU
TECH
PADER
X-Axis
Series1

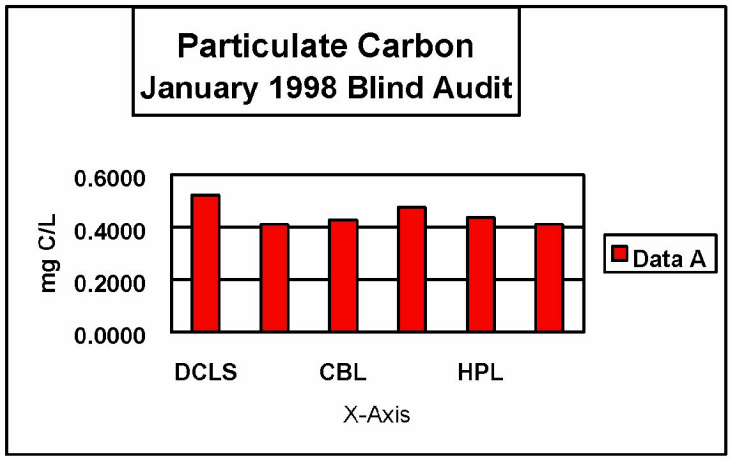

Nitrite+nitrate-N
January 1998 Blind Audit
mg N/L
0.13
0.12
0.11
0.1
DCLS
CBL
ODU
TECH
PADER
X-Axis
Data A

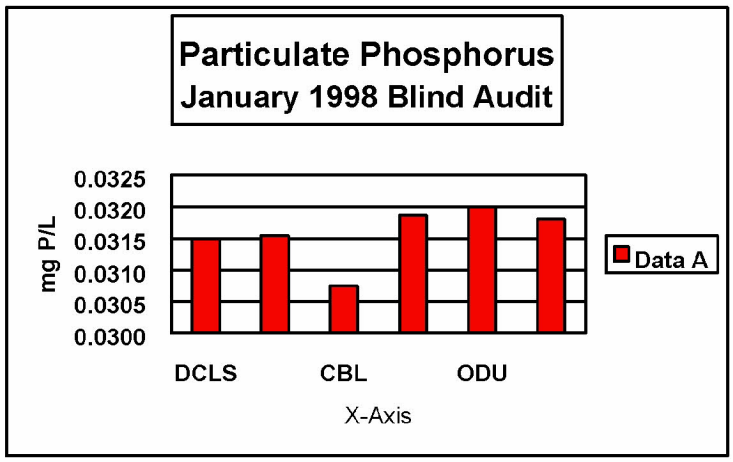

Nitrite+nitrate-N
January 1998 Blind Audit
mg N/L
1.25
1.2
1.15
1.1
1.05
DCLS
CBL
ODU
TECH
PADER
X-Axis
Data A

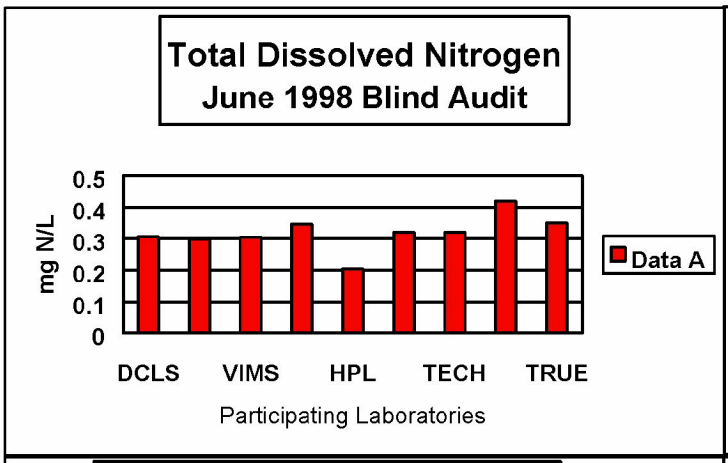
Phosphate
January 1998 Blind Audit
mg P/L
0.05
0.04
0.03
0.02
0.01
0
DCLS
CBL
ODU
TECH
PADER
X-Axis
Data A

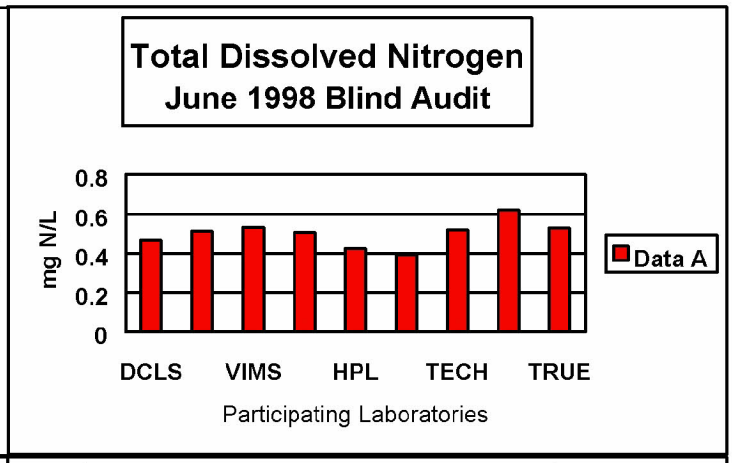
Phosphate
January 1998 Blind Audit
mg P/L
0.34
0.32
0.3
0.28
0.26
DCLS
CBL
ODU
TECH
PADER
X-Axis
Data A

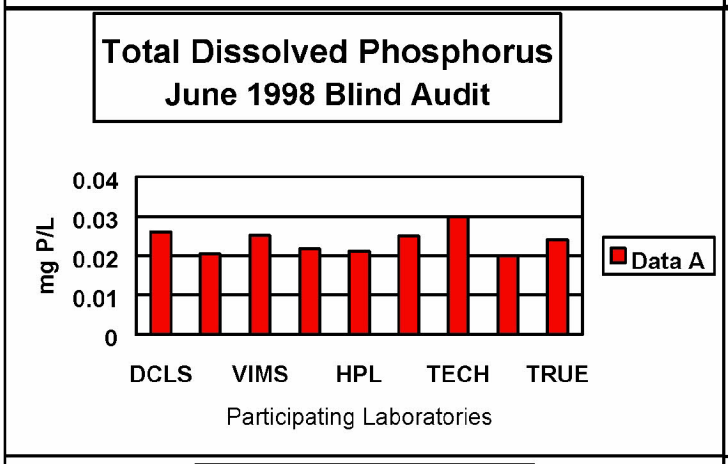

Particulate Nitrogen
January 1998 Blind Audit
0.1000
0.0800
0.0600
0.0400
0.0200
0.0000
mg N/L
DCLS
CBL
HPL
X-Axis
Data A

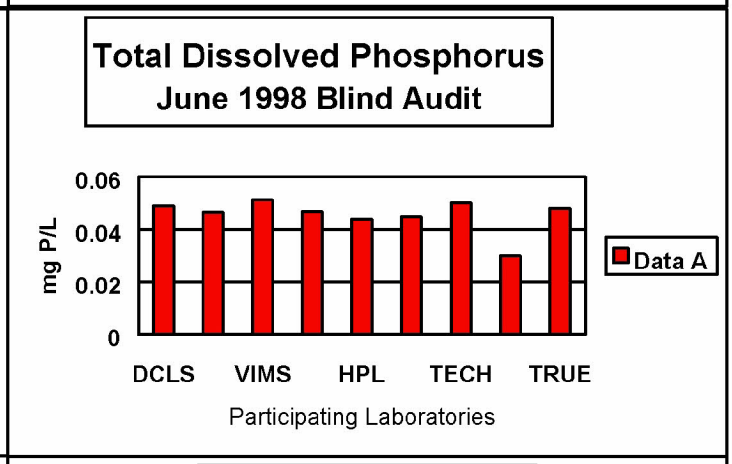

Particulate Carbon
January 1998 Blind Audit
0.6000
0.4000
0.2000
0.0000
mg C/L
DCLS
CBL
HPL
X-Axis
Data A

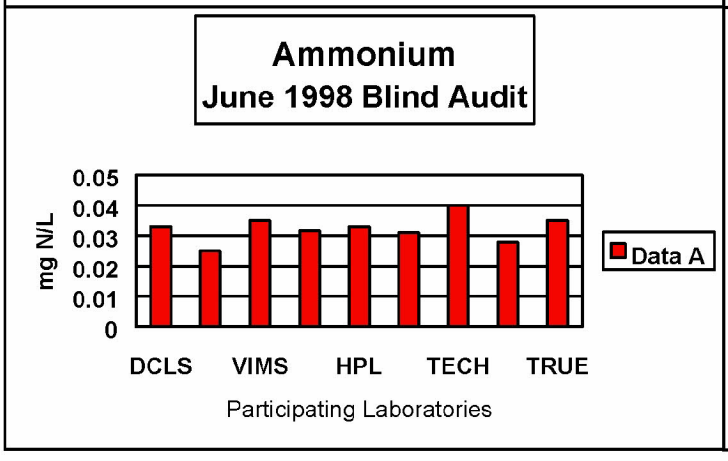

Particulate Phosphorus
January 1998 Blind Audit
0.0325
0.0320
0.0315
0.0310
0.0305
0.0300
mg P/L
DCLS
CBL
ODU
X-Axis
Data A

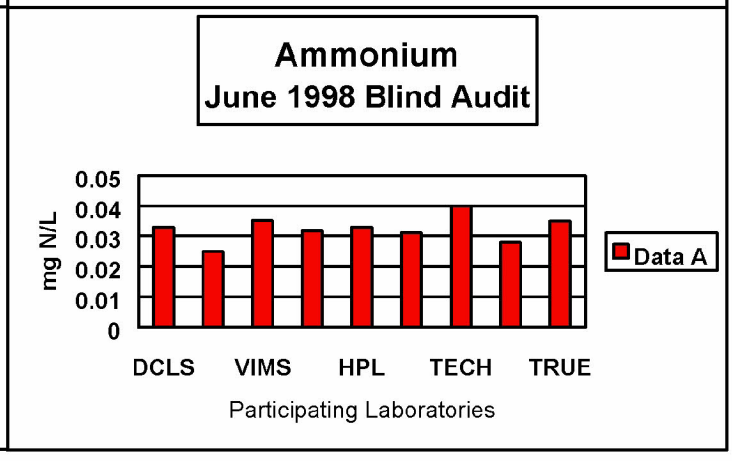

Total Dissolved Nitrogen
June 1998 Blind Audit
mg N/L
0.5
0.4
0.3
0.2
0.1
0
DCLS
VIMS
HPL
TECH
TRUE
Participating Laboratories
Data A

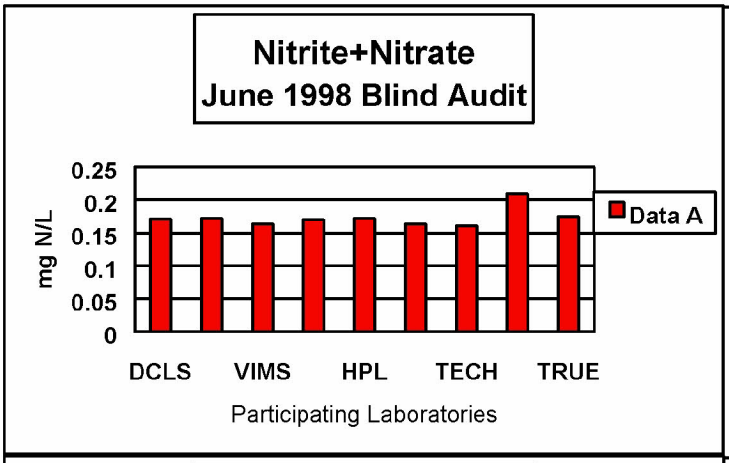

Total Dissolved Nitrogen
June 1998 Blind Audit
mg N/L
0.8
0.6
0.4
0.2
0
DCLS
VIMS
HPL
TECH
TRUE
Participating Laboratories
Data A

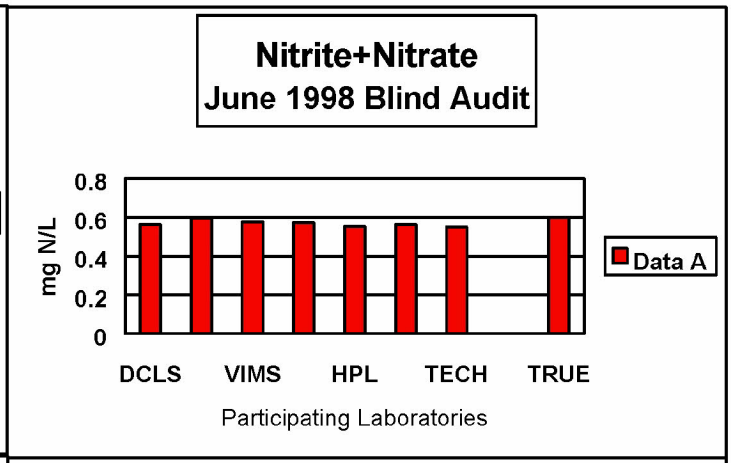

Total Dissolved Phosphorus
June 1998 Blind Audit
mg P/L
0.04
0.03
0.02
0.01
0
DCLS
VIMS
HPL
TECH
TRUE
Participating Laboratories
Data A

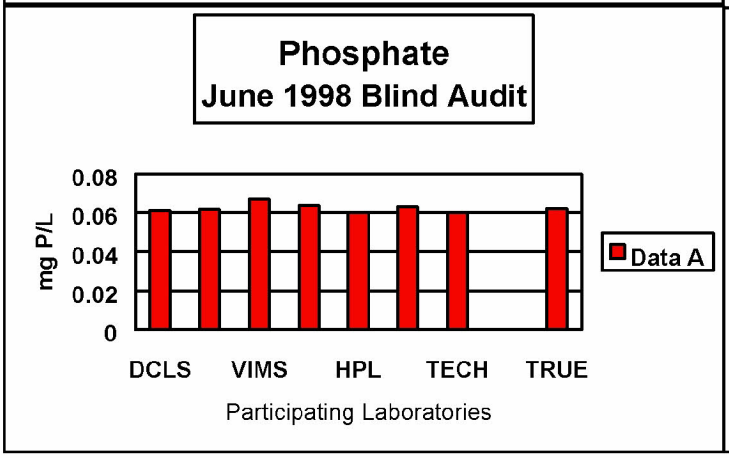

Total Dissolved Phosphorus
June 1998 Blind Audit
mg P/L
0.06
0.04
0.02
0
DCLS
VIMS
HPL
TECH
TRUE
Participating Laboratories
Data A

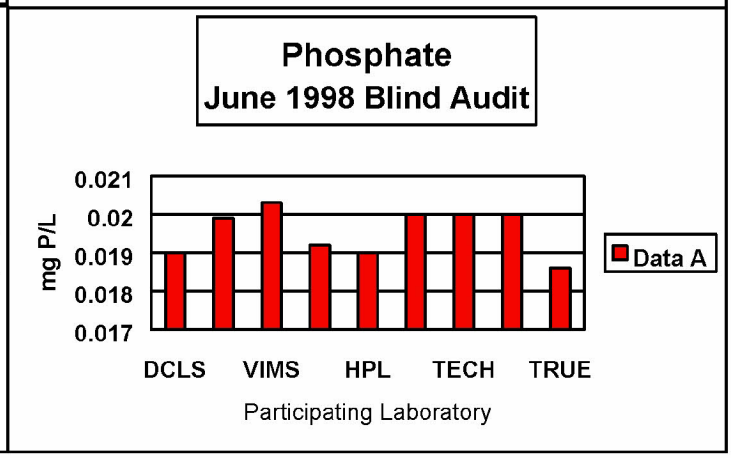

Ammonium
June 1998 Blind Audit
mg N/L
0.05
0.04
0.03
0.02
0.01
0
DCLS
VIMS
HPL
TECH
TRUE
Participating Laboratories
Data A

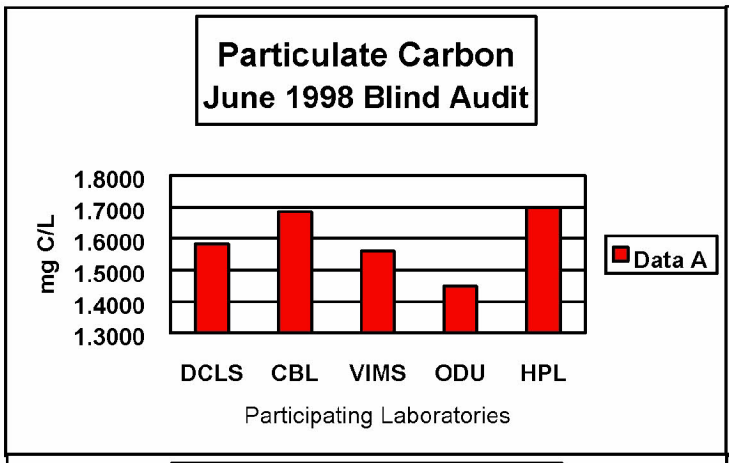

Ammonium
June 1998 Blind Audit
mg N/L
0.05
0.04
0.03
0.02
0.01
0
DCLS
VIMS
HPL
TECH
TRUE
Participating Laboratories
Data A

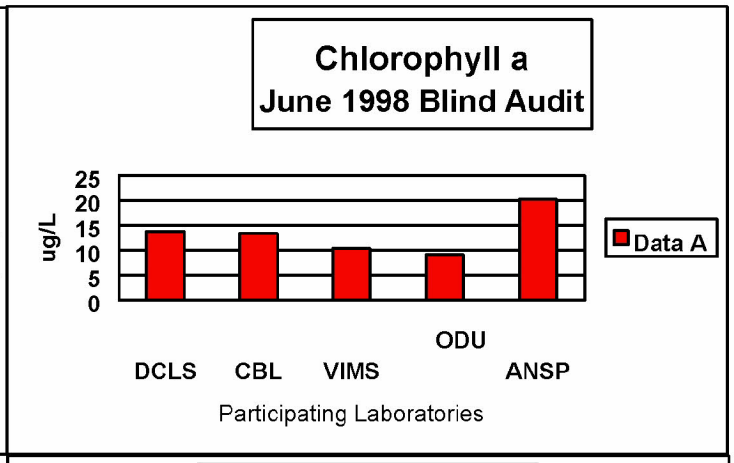

Nitrite+Nitrate
June 1998 Blind Audit
mg N/L
0.25
0.2
0.15
0.1
0.05
0
DCLS
VIMS
HPL
TECH
TRUE
Participating Laboratories
Data A

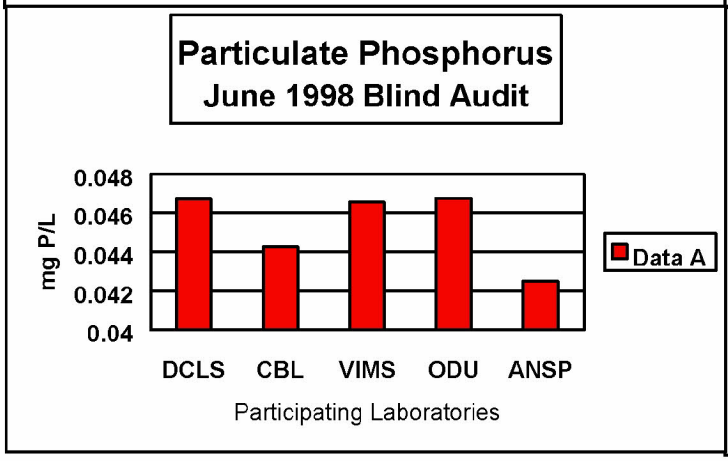

Nitrite+Nitrate
June 1998 Blind Audit
mg N/L
0.8
0.6
0.4
0.2
0
DCLS
VIMS
HPL
TECH
TRUE
Participating Laboratories
Data A

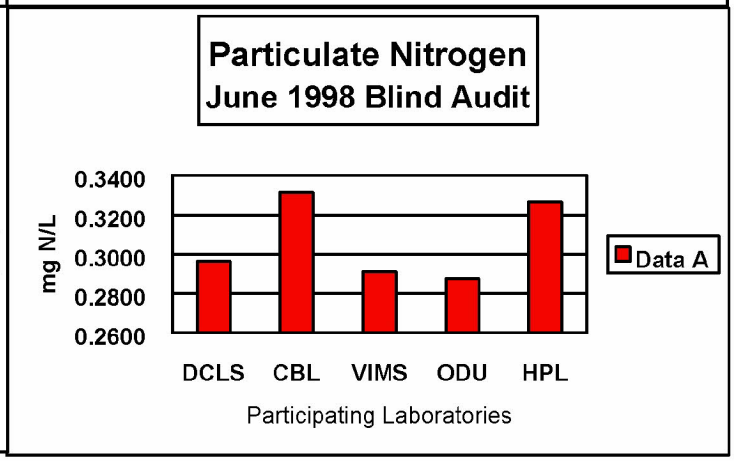

Phosphate
June 1998 Blind Audit
mg P/L
0.08
0.06
0.04
0.02
0
DCLS
VIMS
HPL
TECH
TRUE
Participating Laboratories
Data A


Phosphate
June 1998 Blind Audit
mg P/L
0.021
0.02
0.019
0.018
0.017
DCLS
VIMS
HPL
TECH
TRUE
Participating Laboratory
Data A


Particulate Carbon
June 1998 Blind Audit
mg C/L
1.8000
1.7000
1.6000
1.5000
1.4000
1.3000
DCLS
CBL
VIMS
ODU
HPL
Participating Laboratories
Data A


Chlorophyll a
June 1998 Blind Audit
ug/L
25
20
15
10
5
0
DCLS
CBL
VIMS
ODU
ANSP
Participating Laboratories
Data A


Particulate Phosphorus
June 1998 Blind Audit
mg P/L
0.048
0.046
0.044
0.042
0.04
DCLS
CBL
VIMS
ODU
ANSP
Participating Laboratories
Data A


Particulate Nitrogen
June 1998 Blind Audit
mg N/L
0.3400
0.3200
0.3000
0.2800
0.2600
DCLS
CBL
VIMS
ODU
HPL
Participating Laboratories
Data A